

PREDATORY COMPETENCE OF *DANIO RERIO* (CYPRINIFORMES: CYPRINIDAE) AND *POECILIA RETICULATA* (CYPRINODONTIFORMES: POECILIIDAE) AS BIOCONTROL AGENT OF *AEDES* LARVAE (DIPTERA: CULICIDAE) UNDER THE LABORATORY CONDITION

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ABSTRACT: Predatory potential of Zebrafish, *Danio rerio* (Cypriniformes: Cyprinidae) and Guppy, *Poecilia reticulata* (Cyprinodontiformes: Poeciliidae) were evaluated to control *Aedes* mosquito larvae and thereby manage dengue epidemics in a sustainable, and eco-friendly manner. Under laboratory conditions, size-matched fish of each species were introduced into separate plastic containers containing 2 liters of tap water and 100 third instar larvae of *Aedes*. In addition, different prey combinations were used to assess the comparative predation potential of the fishes. Mosquito prey preferences were assessed using Manly's preference index. Over 24-hours of laboratory conditions, a significant difference ($p < 0.05$) between the predatory efficiency of the studied fish species was reported where the predatory efficiency of *D. rerio* was higher than that of *Po. reticulata*. *D. rerio* showed significantly ($p < 0.05$) higher predation efficiency and prey preference for 2nd instar larvae and pupae of *Aedes* than *Po. reticulata* on the presence of alternative prey. Based on predation efficiency and prey preference, this study suggests that indigenous *Danio rerio* can be evaluated as an alternative species to exotic *Poecilia reticulata* in biological control for more eco-friendly, cost-effective sustainable management of *Aedes* mosquitoes.

Key words: Biological control; larvivorous fish; *Aedes* mosquito; *Aedes aegypti*; dengue

INTRODUCTION

Mosquito-borne diseases are still a major issue in almost every tropical and subtropical country (Sangeetha *et al.* 2021) causing the death of over 700,000 people every year globally (Islam *et al.* 2020). Bangladesh is located in the dengue endemic area of South-East Asia (Rafi *et al.* 2020), and is a suitable habitat for the dengue vector and increased transmission (Mutsuddy *et al.* 2019). In 2000, the country experienced its first dengue epidemic while the worst occurred in 2019, affecting over 100,000 people and resulting in 164

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deaths (Rafi *et al.* 2020). The lack of effective vaccines or drugs against the serotypes (DENV serotypes 1–4) of the dengue virus continues to be a major challenge in controlling dengue epidemics. As a result, various traditional methods for dengue control are being used, with a focus on vector control and patient management (Abir *et al.* 2021). Catching, applying mosquito repellent sprays, using insecticides, using bed nets, indoor residual sprays, and biocontrol agents are the most commonly used control methods (Sangeetha *et al.* 2021). The application of chemical insecticides is a widely used and effective way of mosquito management. In contrast, long-term use of chemical insecticides leads to insecticide resistance and is also dangerous to human health as well as non-target organisms and a major source of pollution (Barik *et al.* 2018). As a result, biological control is an ideal eco-friendly, cost-effective, and effective vector control method in which naturally occurring predators such as amphibians, belostomatids, crustaceans, dytiscid beetles, notonectids, odonates, and larvivorous fish are employed to control the immature or adult phases of vector-borne mosquitoes (Udayanga *et al.* 2019).

However, one of the oldest and most widely used vector control methods is the use of larvivorous fish but chemical insecticides have replaced it (Sangeetha *et al.* 2021). Around the world, more than 253 fish species, both exotic and native, have been considered mosquito biocontrol agents (Sangeetha *et al.* 2021). *Po. reticulata* and *Gambusia affinis* have been promoted for mosquito biological control for a long time and their effectiveness has been reported in numerous cases. However, because of their invasive nature and the inability to achieve desired results in some cases, native larvivorous fish are being promoted as a viable alternative (Aditya *et al.* 2012). Fish reared in artificial containers, such as large domestic tanks and rain-water vessels, have already been used as a biological control alternative in countries such as Brazil, Nicaragua, and Mexico (Cavalcanti *et al.* 2007). The purpose of the present study was to determine the predatory efficiency of native zebrafish and exotic guppies in plastic containers to determine their larvivorous potential in the presence of alternative prey. As a result, determining mosquito preference is a prerequisite before promoting these fish species for biological control (Aditya *et al.* 2012). The presence of alternative prey complicates the interaction, resulting in a predation pattern that is context dependent. Because most mosquito larval habitats are heterogeneous, the predatory ability of fish must be tested in the presence of habitat structural complexity, which influences the outcome of prey-predator interactions (Manna *et al.* 2011). Numerous studies around the world have supported the use of larvivorous fish as biological control agents for vector mosquitoes, the potential of larvivorous fish for controlling *Aedes* larvae has not been adequately evaluated in Bangladesh. Only one study used guppies to control *Culex* mosquitoes but there is no study on *Aedes* (Elias *et al.* 1995). Therefore, the current study was carried out to fill this gap by assessing and comparing the predatory efficiency of native zebrafish and exotic guppies in plastic containers to evaluate their larvivorous potential in the presence of alternative prey for a sustainable, cost-effective, and eco-friendly way to manage dengue epidemics.

MATERIAL AND METHODS

Experimental protocol: The experiment was conducted from November 2021 to February 2022 in the Entomology Research Laboratory and Animal Garden of the Department of Zoology, University of Dhaka, Dhaka, Bangladesh. All experiments were carried out in plastic containers and each parameter was conducted in 3 replicates.

Mosquito rearing

Collection of Aedes eggs: Eggs of *Aedes* species were collected from the Animal Garden in the Department of Zoology, University of Dhaka. Eggs were collected on moist filter paper. Plastic bowls were lined with filter paper and water was added to a depth of 2.5 cm. The egg-collecting bowls were left in the environment for 48 hours. The bowls were then brought to the laboratory and excess water was drained out of the bowls. A batch of eggs was placed inside larval rearing trays with 250 ml tap water.

Rearing of larvae. The eggs hatched into first instar larvae after two days. The larvae were then transferred to small plastic bowls. The larvae were fed daily on yeast. The larvae were gradually molted into second, third, and fourth instar larvae. After a few days, pupae were collected from the plastic bowl and after a few days, adults emerged.

Establishment of Aedes colony: Adult mosquitoes were reared for further experiments. Carbohydrates were generally given as a form of sugar solution. In this rearing process, 10% sucrose was provided as food after a week of adult emergence, a pigeon was placed in each cage to provide a blood meal for 2 hours. Two days following the blood feeding, oviposition cups were kept in each cage where the blood-fed females oviposited. Then the eggs were transferred to a plastic bowl for hatching. Larvae were reared up to the 4th instar by feeding them yeast. Pupae were removed from the larval-rearing bowl and placed into mosquito-rearing cages for the emergence of adults for further rearing.

Identification of Aedes eggs: The eggs were tapering anteriorly and posteriorly and broadly cigar-shaped. The color was dull or matte black or shiny jet-black color. Eggs were identified using the method outlined by Bova *et al.* 2016.

Identification of larvae: The larvae of *Aedes* have thick and short siphons, two siphon feathers, and a comb tooth with the lateral spine (*Aedes aegypti*) or without the lateral spine (*Aedes albopictus*).

Identification of adults: The adult of *Aedes* is characterized by contrasting black-and-white colouration. The scutum has silver scales in the shape of a lyre on a black background (*Ae. aegypti*) or with a median silver-scale line on a black background (*Ae. albopictus*) (Medlock *et al.* 2015) (Fig. 1).

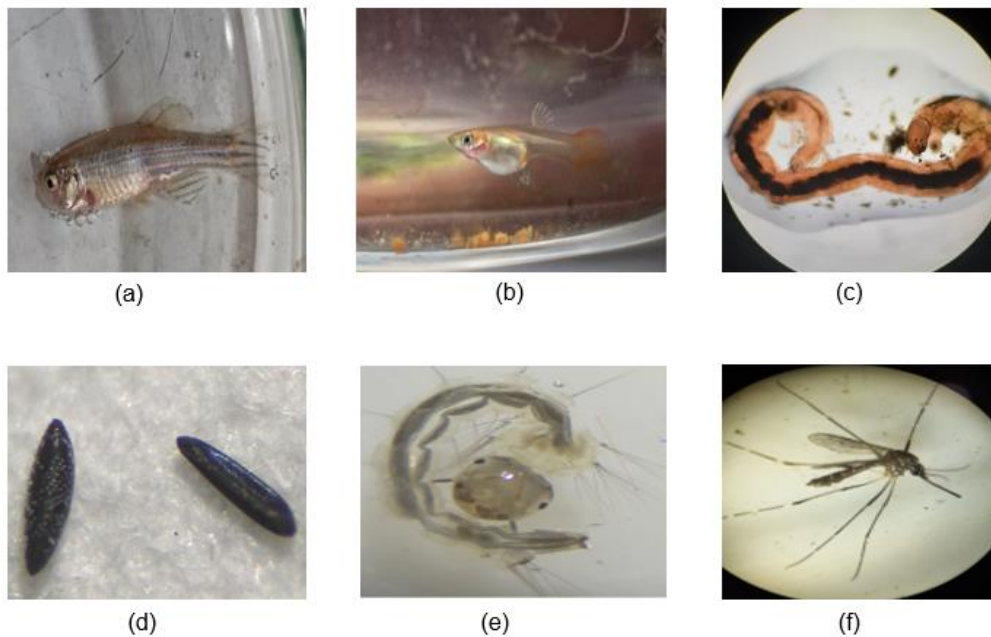


Plate1. Identification of the species used in the experiment; (a) *D. rerio* (b) *Po. reticulata* (c) chironomid larvae (d) eggs of *Aedes* (e) larvae of *Aedes* and (f) adult of *Aedes*.

Collection and maintenance of the fish species: Two fish species *D. rerio* and *Po. reticulata* were collected from a local aquarium shop in Katabon, Dhaka. The fish were then brought to the laboratory and kept in 5 L plastic containers with proper aeration at a density of 8 fish per container. Only female fish were used in the experiment.

Collection of chironomid larvae: Chironomid larvae were collected from the animal garden, Department of Zoology, University of Dhaka. In the laboratory, chironomid larvae were maintained in transparent glass beakers. The beakers contained sewage sediment mixed with sand. In addition, as a supplement crushed fish food was added. The identification was made up to the generic level (Cranston 2019).

Experimental design

Predatory efficiency of fish under laboratory conditions: In the first experiment, the predatory efficiency of the fish was tested under 2 L tap water and 100 prey densities. The 3rd instar larvae of *Aedes* were used separately in this experiment. Three replications were carried out to evaluate the feeding pattern of different individuals of the same fish species. The trials were carried out in transparent plastic jars containing 2 L of tap water (7.06 P^H and 22.6°C temperature) to exclude any other predators. Individual fish were placed in each

container and were held without food for 24 hours prior to each experiment to standardize hunger levels. For each fish, the total length (TL) was measured using normal meter scales. Fish with sizes from 2 cm to 2.8 cm were used in the predatory potential experiment.

In the second experiment, different prey combinations such as larvae of *Aedes* (second, third, and fourth instar), pupae of *Aedes*, chironomid larvae, and fish food were used to assess comparative predation potential on larvae, pupae, and other food. This experiment was conducted under 2 L tap water (7.10 P^H and 20.7°C temperature) and 150 prey densities (25 larvae of each instar, pupae, and other food). Fish with a size from 3.5 cm to 3.8 cm were used in this experiment. Individual fish were placed in each container and were held without food for 24 hours before each experiment to standardize hunger levels. The observations were made for 24 hours. The weight of the fish was measured before and after the experiment with a Kern analytical balance and water quality was measured using a Hach HQD digital multimeter. The consumption of the larvae in laboratory trials was calculated from the difference between the initially introduced mosquito larvae and the remaining larvae. The predatory efficiencies of zebrafish and guppies were calculated by using the following equation (Udayanga *et al.* 2019):

$$\text{Predatory efficiency} = \frac{\left(\frac{\text{Number of prey consumed}}{\text{Number of predator introduced}} \right)}{\text{Total number of prey introduced}} * 100$$

Estimation of prey preference

The prey preference of both fish species was assessed using 2nd, 3rd, and 4th instar larvae of *Aedes* as target prey, chironomid larvae and pupae of *Aedes* as live prey, and fish food as alternative prey. In comparison to other food types, a single fish was allowed to consume the food items at an equal density of mosquito larvae (25 of each food item for a total of 150). The observations were conducted for 24 hours in a plastic container and 2 L of tap water. For each of the fish species, the experiment was conducted 3 times. The predation data were recorded for each fish species, which was then applied to preference analysis. The preference for each food item was calculated by using the following equation (Aditya *et al.* 2012):

$$PP = PC / PA$$

where, PC = proportion of the food item consumed, and PA= proportion of the food item available. For a specific prey type, the selectivity index is then calculated using an equation similar to Manly's a selectivity index.

$$MA = PP / \Sigma PP$$

A deviation from the expected value of 0.17 (for equal mosquito density) was used to determine the prey preference. As different food items were available, any value above these will indicate a relative preference whereas any value below will indicate a relative avoidance of mosquito larvae. A t test (one-tailed) was used to justify significant relative avoidance and relative preference for the food items with values less or more than expected.

Data analysis: SPSS (version 25.0) and GraphPad Prism were used to analyse the data. The results of this study were presented as mean \pm standard error. An independent sample t-test was used to assess the significant difference between the larval consumption of *D. rerio* and *Po. reticulata*. The Pearson correlation coefficient was used to assess the relationship between the morphology of fish (mass and total length) and predatory efficiency. To determine the variation in the food consumption of fish one way ANOVA was used and to assess which food groups were significantly consumed Tukey's Post Hoc test was used.

RESULTS AND DISCUSSION

Total number of larvae consumed and predatory efficiency: The consumption of total *Aedes* larvae (3rd instar) and predatory efficiency of the studied fish species are shown in Table 1. *D. rerio* showed the highest predation rates within 24 hours, consuming 63.67 \pm 3.17 larvae, and the predatory efficiency was 63.67 \pm 3.17%, whereas *Po. reticulata* showed the lowest predation rates, consuming 33.33 \pm 4.37 larvae, and the predatory efficiency was 33.33 \pm 4.37%. There was a significant ($P < 0.05$) difference between the larval consumption and predatory efficiency of *D. rerio* and *Po. reticulata*. The predatory efficiency of *D. rerio* was 1.91 times higher than that of *Po. reticulata*.

Table 1. Consumption of 3rd instar larvae of *Aedes* and predatory efficiency within 24 hours

Fish species	Larvae consumed by a single fish ^a	Predatory efficiency (%) ^a
<i>D. rerio</i>	63.67 \pm 3.17*	63.67 \pm 3.17*
<i>Po. Reticulata</i>	33.33 \pm 4.37	33.33 \pm 4.37

^a Mean \pm SE

* $p < 0.05$ in independent samples test between *D. rerio* and *Po. reticulata* in terms of larval consumption and predatory efficiency

Predictors of larvae consumption: Table 2 shows the total length and weight of the fish that may affect the consumption of *Aedes* larvae and predatory efficiency. The highest predatory efficiency was found in *D. rerio* (63.67 \pm 3.17) which had the highest values for body weight (0.17 \pm 0.12 g) and total length (2.73 \pm 0.03 cm) whereas *Po. reticulata* had the smallest body weight (0.08 \pm 0.003 g) and total length (2.03 \pm 0.03 cm) and a predatory efficiency of 33.33 \pm 4.37%.

Table 2. Morphological features of *D. rerio* and *Po. reticulata*

Fish species	Life stage	Weight in g ^a	Length in cm ^a
<i>D. rerio</i>	Adult	0.17 ± 0.12*	2.73 ± 0.03*
<i>Po. reticulata</i>	Adult	0.08 ± 0.003	2.03 ± 0.03

^a Mean ± SE

* $p < 0.05$ in independent samples t test between *D. rerio* and *Po. reticulata* for their weight and length

The Pearson correlation analysis indicated that two predictors (weight and total length) significantly influenced ($P < 0.05$) the consumption of larvae and predatory efficiency of *D. rerio* and *Po. reticulata*. There was a positive correlation between the weight of fish and predatory efficiency (Fig. 1a) and between the total length of fish and predatory efficiency (Fig. 1b).

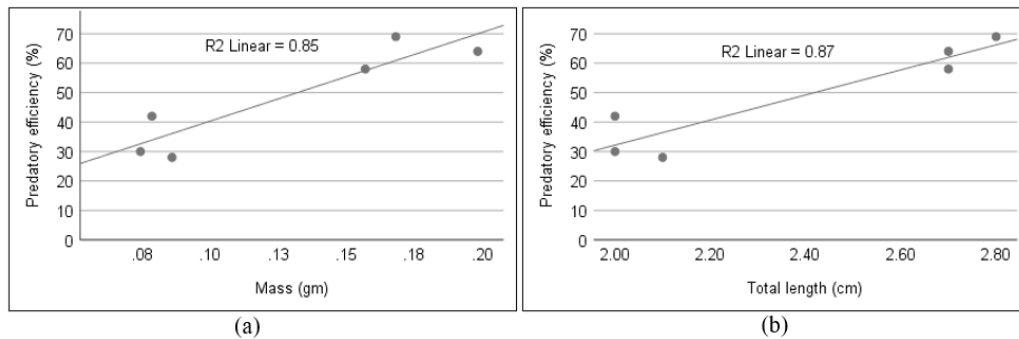


Fig. 1. Correlation between predatory efficiency and morphology of *D. rerio* and *Po. reticulata*. (a) Predatory efficiency and mass and (b) predatory efficiency and total length of *D. rerio* and *Po. reticulata*.

Fig. 2a shows that *D. rerio* consumed 367 larvae per day and *Po. reticulata* consumed 421 larvae per day in terms of mass. While *D. rerio* consumed 23 larvae per day and *Po. reticulata* consumed 16 larvae per day in terms of length (Fig. 2b).

Predatory efficiency on mosquito larvae and pupae in the presence of alternative food: The variation in the different food consumption of zebrafish ($F(5,12) = 22.786, p < 0.01$) and guppies ($F(5,12) = 9.65, p < 0.01$) differed significantly as suggested by the result of one-way ANOVA. Fig. 3a shows that zebrafish consumed significantly more 2nd and 3rd instar larvae than pupae, chironomid larvae, and fish foods and the fish consumed significantly more 4th instar larvae than fish food. Fig. 3b shows that guppies significantly consumed more 2nd and 3rd instar larvae than pupae and fish food and significantly consumed 4th instar and chironomid larvae over pupae. There was no significant

difference between the consumption of mosquito larvae (2nd, 3rd, and 4th instars) and chironomid larvae.

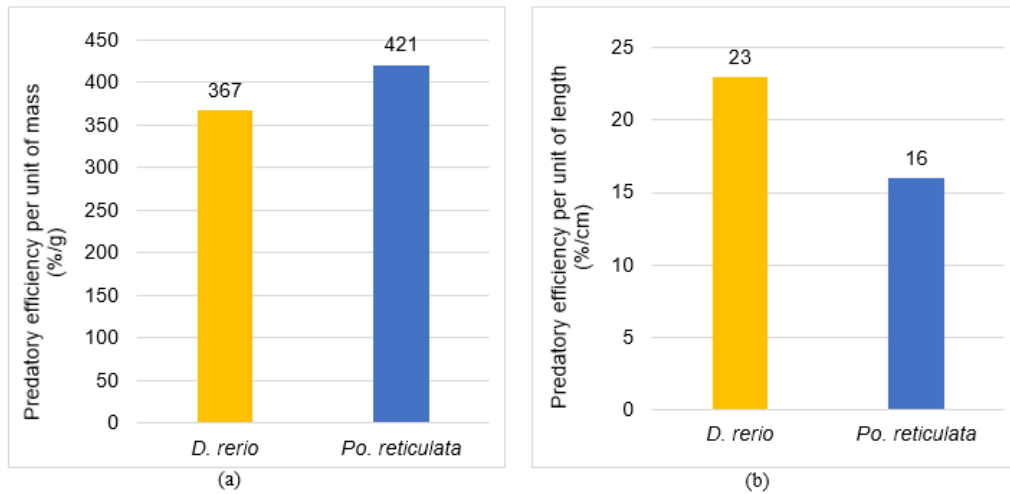


Fig. 2. Predatory efficiency of *D. rerio* and *Po. reticulata*. Predatory efficiency in terms of (a) per unit of mass and (b) per unit of length. The efficiency was measured as a percentage, while mass was measured as grams and length was measured as centimeters.

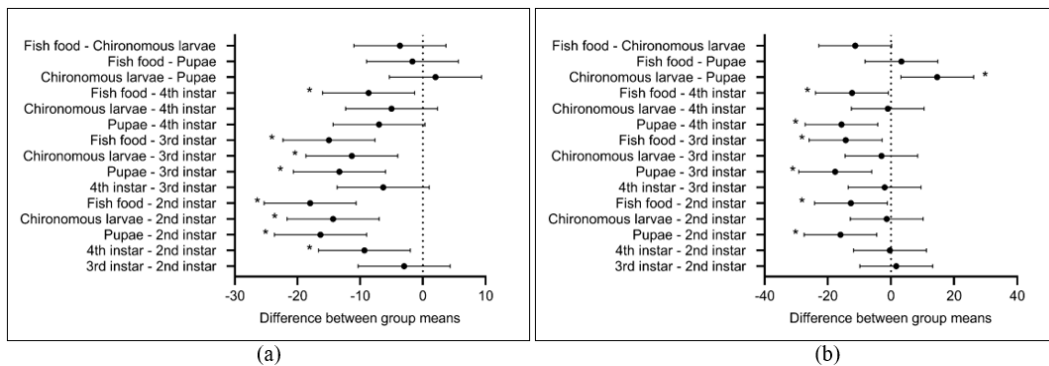


Fig. 3. Comparison of different food consumption within a 24-hour period by (a) *D. rerio* and (b) *Po. reticulata*. The dot with a horizontal bar indicates the mean \pm 95% confidence interval. The significant differences between food groups are indicated by asterisks (one way ANOVA $p < 0.05$ followed by Tukey's Post Hoc test).

The comparative predation efficiency of guppies and zebrafish is shown in Fig. 4. *D. rerio* showed significantly ($p < 0.05$) higher predation efficiency on 2nd instar larvae and pupae than *Po. reticulata*. The predation efficiency of both fish for the 3rd instar larvae was equal. *Po. reticulata* consumed more 4th instar larvae than *D. rerio* and *Po. reticulata* showed the highest predation efficiency on chironomid larvae compared to *D. rerio*.

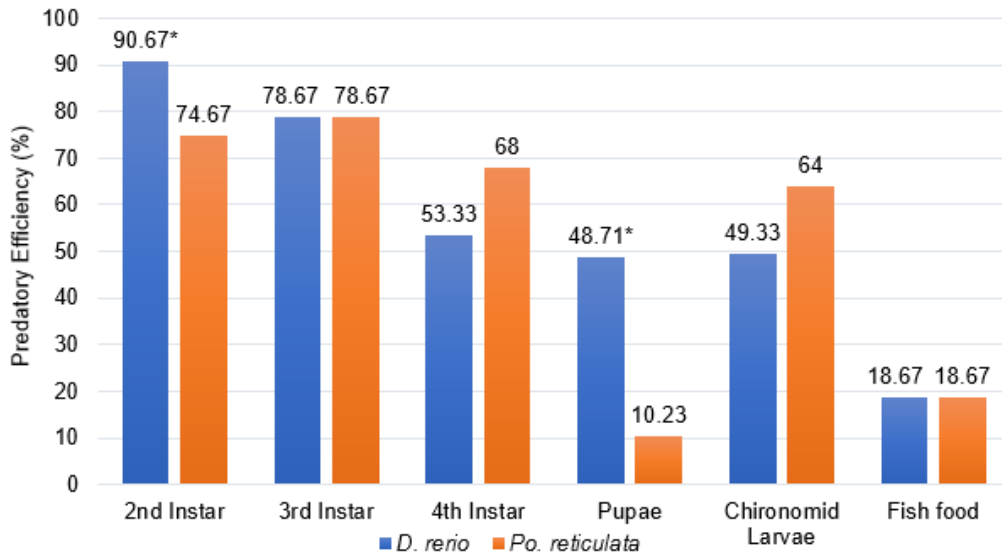


Fig. 4. Predatory efficiency of *D. rerio* and *Po. reticulata* on the presence of alternative foods. A significant difference is indicated by an asterisk (independent samples t test $p < 0.05$).

Prey preference of D. rerio and Po. reticulata: Fig. 4 depicts the different numbers of food items consumed by *D. rerio* and *Po. reticulata*. They consumed all food types but the amounts were different from each other. At equal mosquito densities, Table 3 shows that *D. rerio* and *Po. reticulata* exhibited a higher preference for all food items than expected values. *D. rerio* showed a significantly higher preference for 2nd instar larvae and pupae than *Po. reticulata*.

Table 3. Selectivity index shown by *D. rerio* and *Po. reticulata* for the different food items at an equal density of *Aedes* larvae (25:25)

Food items	2 nd instar	3 rd instar	4 th instar	Pupae	Chironomid larvae	Fish food
Expected selectivity value	0.17	0.17	0.17	0.17	0.17	0.17
<i>D. rerio</i>						
Mean ± SE	0.33±0.05*	0.33±0.16	0.33±0.08	0.33±0.02*	0.3±0.15	0.3±0.08
t value	3.5	1.02	2.0	7.0	1.09	2.11
<i>Po. reticulata</i>						
Mean ± SE	0.34±0.19	0.33±0.15	0.33±0.11	0.33±0.23	0.3±0.10	0.3±0.10
t value	0.85	1.06	1.41	0.70	1.57	1.50

* $p < 0.05$ in independent samples t test between *D. rerio* and *Po. reticulata* for each food item

Although chemical insecticides are the most widely used method for mosquito control, the long-term use of insecticides has an adverse effect on human health, non-target organisms, and the environment and causes vector resistance (Barik *et al.* 2018). As a result, a more effective, environmentally friendly, and cost-effective method is the use of natural predators to control *Aedes* mosquitoes (Kalimuthu *et al.* 2017). The study was conducted to evaluate the larvivorous potential of *D. rerio* and *Po. reticulata* in the presence of alternative prey.

D. rerio showed the highest larval consumption and predatory efficiency of 63.67% over *Aedes* larvae, while *Po. reticulata* consumed the least in the laboratory. Parallel to the current study several other studies showed that indigenous fish are more effective in controlling *Ae. aegypti* larvae than exotic *Po. reticulata* (Ranathunge *et al.* 2021). Five native fish, *Astyanax fasciatus*, *Lepisosteus tropicus*, *Ictalurus meridionalis*, *Brycon guatemalensis*, and *Poecilia sphenops* were very effective biocontrol agents of *Ae. aegypti* larvae in a domestic cement tank in Mexico (Martinez-Ibarra *et al.* 2002). Several other studies showed that *D. rerio* demonstrated promising predation potential against *Culex* mosquitoes and consumed an average of 62 larvae at 100 prey density (Sangeetha *et al.* 2021, Kamatchi *et al.* 2016). *D. rerio* consumed 52 fourth instar larvae of *Anopheles* per day in laboratory conditions (Chandra *et al.* 2008). The above study showed that the predatory potential of larvivorous fish was higher in environmental conditions than in laboratory settings. In the current study, zebrafish consumed 63.67% larvae in laboratory settings which may increase under field conditions.

In the present study, *D. rerio* consumed 367 *Aedes* larvae per gram of weight per day and *Po. reticulata* consumed 421 *Aedes* larvae per gram per day (Fig. 2a). In terms of length, *D. rerio* consumed 23 larvae per day, and *Po. reticulata* consumed 16 larvae per day (Fig. 2b). The estimation of the number of larvae consumed per unit mass and per unit length by each fish makes it possible to compare the predatory potential of the fish species and found that female *Betta splendens* consumed 406 to 523 larvae per gram per day, female *Astyanax fasciatus* consumed 281 to 349 larvae per gram per day, and male *Trichogaster trichopteros* consumed 117 to 200 larvae per gram per day. These fish proved to be the most effective predators of the *Ae. aegypti* larvae (Cavalcanti *et al.* 2007). In the present study, the weight and size of the fish were positively correlated with larval consumption which was supported by earlier studies (Wani and Shrivastava 2021). The results showed that *D. rerio* significantly consumed more 2nd instar larvae and pupae of *Aedes* than guppies. When other foods are available, *D. rerio* prefers to eat the earlier larval instar over the later instar and prefers mosquito larvae and pupae over other foods.

Another study showed that *Aplocheilichthys panchax* preferred mosquito larvae over alternative prey (Manna *et al.* 2011). It was found that *D. rerio* reared in rice fields filled with mosquitoes had tremendously reduced larval and pupal densities in the rice field (Chandra *et al.* 2008). It was also reported that zebrafish showed higher predation potential against mosquito larvae in the pond and drain water than guppies. *Trichogaster fasciata*, native fish of Bangladesh commonly known as banded gourami showed the higher predation potential against mosquito larvae than guppies and the fish can also survive in drain water (Lhamo and Rashid 2018).

For the mosquito and non-mosquito preference test involving *Aedes* larvae, pupae, chironomid larvae, and fish food combined with *D. rerio* and *Po. reticulata* showed a higher preference for all food items than expected values at equal mosquito densities. *D. rerio* showed a significantly higher preference for 2nd instar larvae and pupae than *Po. reticulata*. From the results, it is evident that there was no significant difference between the consumption of mosquito larvae and chironomid larvae by guppies and it consumed more chironomid larvae than *Aedes* pupae. Indigenous larvivorous fish have been found to be more effective in vector control operations, arguing for their widespread use (Sangeetha *et al.* 2021).

CONCLUSION

This study determined the predatory efficiency and feeding preference of *D. rerio* and *Po. reticulata*. The predatory efficiency of *D. rerio* is significantly higher than that of *Po. reticulata* and *D. rerio* was significantly more effective in preying upon 2nd instar larvae and pupae of *Aedes* than *Po. reticulata* when alternative prey was available. Therefore, *D. rerio* can be introduced during the start of the vector season to control the earlier stage of *Aedes* larvae and introduced at the end of the vector season to control the pupal stage of *Aedes* mosquitoes. The present study suggests that *D. rerio* can be evaluated as an alternative species to *Po. reticulata* for the management of *Aedes* mosquitoes. Further studies are recommended to evaluate the survival rate and predatory potential of *D. rerio* under different semi-environmental and environmental settings.

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